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Netamines O–S, Five New Tricyclic Guanidine Alkaloids from the Madagascar Sponge *Biemna laboutei*, and Their Antimalarial Activities

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In our continuing program to isolate new compounds from the Madagascar sponge *Biemna laboutei*, five new tricyclic guanidine alkaloids, netamines O – S (**1–5**, resp.), have been identified together with the known compounds netamine E (**6**) and mirabilin J (**7**). The structures of all new netamines were assigned on the basis of spectroscopic analyses. Their relative configurations were established by analysis of ROESY data and comparison with literature data. Netamines O, P, and Q, which were isolated in sufficient quantities, were tested for their cytotoxic activities against KB cells and their activities against the malaria parasite *Plasmodium falciparum*. Netamines O and Q were found to be moderately cytotoxic. Netamines O, P, and Q exhibited antiplasmodial activities with IC_{50} values of 16.99 ± 4.12 , 32.62 ± 3.44 , and 8.37 ± 1.35 μ M, respectively.

Introduction. – Tricyclic guanidine compounds, bearing a tricyclic 2-amino-1,3-diazaoctahydroacenaphthylene skeleton, like ptilocaulins, mirabilins, and netamines, are restricted to marine sponges of the order Poecilosclerida [1–11] and can be grouped on the basis of their degree of oxidation and C=C bond position with pyrimidine, $\Delta^{7,8}$ -, $\Delta^{8,8a}$ -, $\Delta^{8a,8b}$ -unsaturated, or saturated heterocycles. Many of these alkaloids were reported to possess noteworthy biological activities, *i.e.*, cytotoxic [1][4][8][9][12], antibacterial [1][7], antifungal [3], antimalarial [3][11], and antiprotozoal activities [3].

In 2006, we reported the isolation and structure elucidation of seven alkaloids, named netamines A–G, from the Madagascar sponge *Biemna laboutei* HOOPER, 1996 collected twice in May 2004 near Sainte Marie Island, and once in January 2005 at Itampule [9]. From yet another collection of the sponge in October 2009 in Salary Bay (*ca.* 100 km north of Tulear), we isolated several additional tricyclic guanidine alkaloids, including the known netamine G, mirabilins A, C, and F, and seven new

tricyclic alkaloids, netamines H–N [11]. All the latter new compounds isolated from *B. laboutei* possess a 5,6,8b-triazaperhydroacenaphthylene skeleton. Except for netamines A–D that possess a saturated ring system, netamine E shows a C(8a)=C(8b) bond, netamines K–N have a C(8)=C(8a) bond, and netamines F–J contain a pyrimidine ring system.

In this article, we describe the isolation, structure elucidation, and biological characterization of a series of five new tricyclic guanidines, netamines O–S (**1–5**), along with the known compounds netamine E and mirabilin J. All these compounds, isolated from the 2009 Salary Bay sponge, show a C(8a)=C(8b) bond.

Results and Discussion. – *Structure Elucidation.* Chemical fractionation of the crude extract, using a sequence of medium pressure liquid chromatography over silica gel or reversed phase, followed by repetitive reversed-phase preparative HPLC, yielded new (**1–5**) and known (**6** and **7**) compounds. The known compounds, netamine E (**6**) and mirabilin J (**7**) were identified by comparison with published spectroscopic data [8][9] revised by Snider [10]. For the known isolated compound netamine E (**6**), the $[\alpha]_D^{25}$ value of +37.0 ($[\alpha]_D^{25} = +35.0$ [9]) establishes the absolute configuration as shown in the *Figure*. Although the optical rotations of all co-isolated compounds have the same algebraic sign as netamine E, we were careful not to conclude for compounds **1–5** and favored to stick to our ROESY data and the relative configuration.

Netamine O (**1**) was obtained as pale yellow oil. The molecular formula was established by HR-ESI-MS to be $C_{17}H_{28}N_3$. Analysis of the 1D and 2D 1H -, ^{13}C -, and ^{15}N -NMR data for **1** (*Table 1*) revealed resonances and correlations consistent with those of a tricyclic guanidinium system with five C=C bond equivalents, three CH_2 ($\delta(C)$ 30.9, 34.1, and 34.2) and four CH groups (34.8, 38.8, 39.9, and 53.0), a tetra-

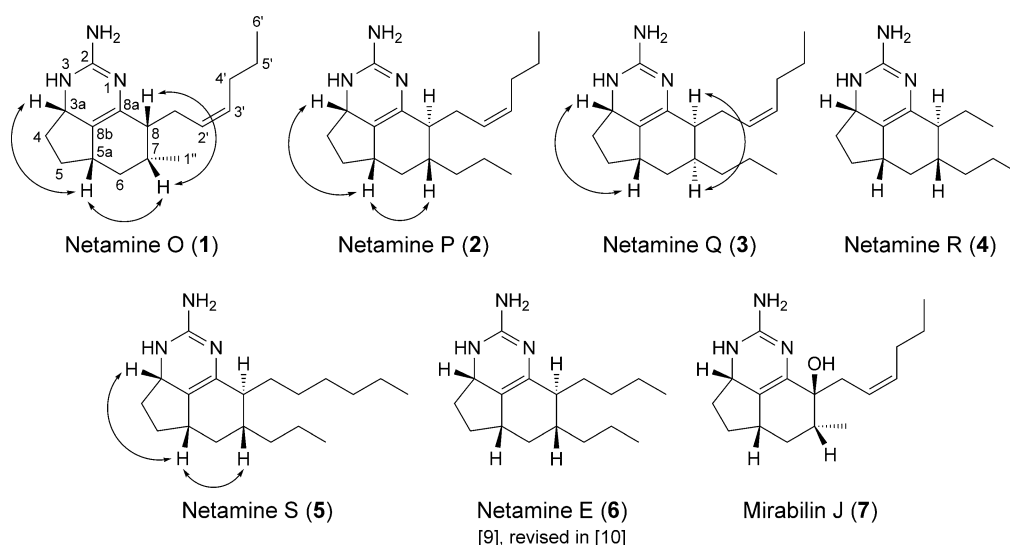


Figure. Structures of the tricyclic guanidine alkaloids netamines O–S (**1–5**, resp.), netamine E (**6**), and mirabilin J (**7**). Relative configurations were determined by 1H , 1H -ROESY ($H \leftrightarrow H$) correlations.

Table 1. ^1H - and ^{13}C -NMR Data (500 and 125 MHz, resp.; in CD_3OD) for Netamine O (**1**). δ in ppm, J in Hz.

Position	$\delta(\text{H})$	$\delta(\text{C})$	COSY	HMBC (H \rightarrow C)
1	12.10 ^a)	—	—	—
2	—	154.3	—	—
$\text{H}_2\text{N}-\text{C}(2)$	6.49 ^a)	—	—	—
3	9.85 ^a)	—	—	—
3a	4.23–4.28 (<i>m</i>)	53.0	—	5, 8b
4	2.23–2.25 (<i>m</i>)	34.1	5	8b
5	1.18–1.21 (<i>m</i>), 1.70–1.76 (<i>m</i>)	34.2	4, 5a	3a, 8b
5a	2.46–2.52 (<i>m</i>)	38.8	5, 6	5, 8a, 8b
6	1.21–1.23 (<i>m</i>)	30.9	5a, 7	—
7	1.98–2.05 (<i>m</i>)	34.8	1'', 6	1', 1''
8	2.31–2.35 (<i>m</i>)	39.9	—	1', 1''
8a	—	129.4	—	—
8b	—	120.6	—	—
1'	2.26–2.30 (<i>m</i>)	26.9	8	2', 8, 8a
2'	5.34–5.41 (<i>m</i>)	129.1	3'	3'
3'	5.37–5.43 (<i>m</i>)	131.8	2', 4'	4', 5'
4'	1.96–2.01 (<i>m</i>)	30.7	3', 5'	3', 6'
5'	1.34–1.41 (<i>m</i>)	23.9	4', 6'	3', 6'
6'	0.92 (<i>t</i> , $J = 7.1$)	14.3	5'	4', 5'
1''	1.07 (<i>d</i> , $J = 7.0$)	19.4	7	7, 8

^a) Recorded in DMF.

substituted C=C bond (120.6 and 129.4), a guanidine-like C-atom (154.3), and three N-atoms ($\delta(\text{N})$ 78.1, 89.1, and 103.4). The HMB correlations of H–C(5a) and $\text{CH}_2(1')$ with C(8a) ($\delta(\text{C})$ 129.4) and of H–C(3a), $\text{CH}_2(4)$, $\text{CH}_2(5)$, and H–C(5a) with C(8b) (120.6) indicated a $\Delta^{8a,8b}$ -unsaturated tricyclic ring system like in netamine E [9]. In addition, HMBC data clearly indicated that the heterocyclic ring system is substituted at C(7) ($\delta(\text{C})$ 34.8) and C(8) (39.9) by two alkyl groups summing up a total of seven C-atoms. One substituent is a (2*Z*)-hex-2-en-1-yl group identified by a C(1') to C(6') correlation sequence. Its linkage to the C(8)-atom was determined by the HMB correlation of $\text{CH}_2(1')$ with C(8). The geometry of the C=C bond was confirmed by a NOE correlation between $\text{CH}_2(1')$ ($\delta(\text{H})$ 2.26–2.30) and $\text{CH}_2(4')$ (1.96–2.01). Therefore, the second substituent has to be a Me group ($\delta(\text{H})$ 1.07) attached to C(7) on the basis of the COSY correlation of Me(1'') with H–C(7). Moreover, the relative configuration of the four stereogenic centers at C(3a), C(5a), C(7), and C(8) was determined by ROESY correlations between H–C(3a) ($\delta(\text{H})$ 4.23–4.28) and H–C(5a) (2.46–2.52), H–C(5a) and H–C(7) (1.98–2.05), and H–C(7) and H–C(8) (2.31–2.35). Thus, the four H-atoms were deduced to be at the same side of the tricyclic ring system and the two side chains were established to be *cis* to each other. Therefore, the relative configuration of Netamine O (**1**) is as shown in the *Figure*.

Netamine P (**2**) was isolated as white amorphous solid and has a molecular formula of $\text{C}_{19}\text{H}_{32}\text{N}_3$, which was suggested by HR-ESI-MS. Analysis of the NMR data (*Tables* 2 and 3) indicated again a guanidinium moiety, *i.e.*, the presence of three CH_2 ($\delta(\text{C})$ 30.3,

Table 2. ¹H-NMR Data (in CD₃OD) of Netamines O–S (**1**–**5**, resp.). δ in ppm, J in Hz.

Position	1 ^{a)}	2 ^{a)}	3 ^{b)}	4 ^{a)}	5 ^{a)}
3a	4.23–4.28 (m)	4.26–4.30 (m)	4.23–4.26 (m)	4.25–4.28 (m)	4.25–4.30 (m)
4	2.23–2.25 (m)	1.65–1.69 (m), 2.16–2.23 (m)	1.70–1.76 (m), 2.22–2.25 (m)	1.65–1.72 (m), 2.16–2.24 (m)	1.63–1.69 (m), 2.16–2.23 (m)
5	1.18–1.21 (m), 1.70–1.76 (m)	1.28–1.34 (m), 1.96–2.02 (m)	1.20–1.23 (m)	1.22–1.29 (m), 1.98–2.03 (m)	1.97–2.03 (m)
5a	2.46–2.52 (m)	2.36–2.42 (m)	2.44–2.50 (m)	2.32–2.38 (m)	2.37–2.45 (m)
6	1.21–1.23 (m)	2.09–2.13 (m), 0.73–0.80 (m)	1.17–1.19 (m), 1.78–1.83 (m)	0.74–0.79 (m), 2.06–2.12 (m)	0.73–0.80 (m), 2.09–2.14 (m)
7	1.98–2.05 (m)	1.52–1.56 (m)	1.83–1.88 (m)	1.53–1.59 (m)	1.54–1.60 (m)
8	2.31–2.35 (m)	1.97–2.00 (m)	2.38–2.41 (m)	1.93–1.99 (m)	1.92–1.97 (m)
1'	2.26–2.30 (m)	2.30–2.36 (m), 2.52–2.58 (m)	2.24–2.27 (m)	1.71–1.78 (m)	1.57–1.65 (m), 1.70–1.73 (m)
2'	5.34–5.41 (m)	5.27–5.33 (m)	5.36–5.41 (m)	0.81 (t, J=7.6)	1.15–1.20 (m), 1.25–1.27 (m)
3'	5.37–5.43 (m)	5.48–5.54 (m)	5.37–5.43 (m)	–	1.27–1.34 (m)
4'	1.96–2.01 (m)	2.04–2.08 (m)	1.98–2.02 (m)	–	1.27–1.34 (m)
5'	1.34–1.41 (m)	1.36–1.44 (m)	1.34–1.41 (m)	–	1.27–1.34 (m)
6'	0.92 (t, J=7.1)	0.93 (t, J=7.1)	0.91 (t, J=7.3)	–	0.90 (t, J=6.9)
1''	1.07 (d, J=7.0)	1.16–1.23 (m), 1.60–1.63 (m)	1.30–1.32 (m), 1.42–1.47 (m)	1.14–1.22 (m)	1.17–1.22 (m)
2''	–	1.24–1.32 (m), 1.48–1.52 (m)	1.40–1.42 (m)	1.27–1.35 (m)	1.27–1.34 (m), 1.46–1.53 (m)
3''	–	0.94 (t, J=7.0)	0.95 (t, J=6.9)	0.94 (t, J=7.1)	0.94 (t, J=7.1)

^{a)} Recorded at 500 MHz. ^{b)} Recorded at 600 MHz.

34.0, and 36.3) and four CH groups (37.6, 38.9, 42.2, and 54.2), a tetrasubstituted C=C bond (120.0 and 128.6), a deshielded sp²-hybridized C-atom (159.7), and three N-atoms (δ(N) 78.1, 89.1, and 102.2). Relevant correlations between the ¹H- and ¹³C-NMR spectra for netamines O and P (**1** and **2**; resp.) supported a tricyclic guanidine-like arrangement with a C(8a)=C(8b) bond. However, the differences observed between **1** and **2** in the ¹H- and ¹³C-NMR spectra indicated that the Me group at C(7) of **1** is replaced by a Pr group at C(7) in **2**. The latter group was established by COSY correlations observed between Me(3'') (δ(H) 0.94), CH₂(2'') (1.24–1.32 and 1.48–1.52), CH₂(1'') (1.16–1.23 and 1.60–1.63), and H–C(7) (1.52–1.56). NOESY correlations between H–C(5a) and H–C(3a) and H–C(7) determined all three to be positioned at the same side of the molecule. In addition, NOESY correlations recorded in (D₇)DMF suggested that H–C(3a) (δ(H) 4.26–4.30), H–C(5a) (2.36–2.42), and H–C(7) (1.52–1.56) are located at the same side, while H–C(8) (1.97–2.00) is located at the opposite side of the molecule. Thus, the side chains of **2** are *trans* to each other.

Netamine Q (**3**) was obtained as white amorphous solid. The MS spectrum suggested the same formula C₁₉H₃₂N₃ as for **2**, implying an isomeric structure. Comparison of their 1D- and 2D-NMR data, reinforced by a detailed analysis of the 2D-NMR spectra, also revealed that the two molecules have the same constitution and

Table 3. ^{13}C -NMR Data (in CD_3OD) of Netamines O–S (**1**–**5**, resp.). δ in ppm.

Position	1 ^{a)}	2 ^{a)}	3 ^{b)}	4 ^{a)}	5 ^{a)}
2	154.3	159.7	154.4	154.8	154.7
3a	53.0	54.2	53.0	54.0	54.1
4	34.1	34.0	34.1	34.1	34.0
5	34.2	30.3	31.1	30.4	30.4
5a	38.8	37.6	38.9	37.6	37.7
6	30.9	36.3	32.5	36.5	36.6
7	34.8	38.9	38.4	37.7	38.4
8	39.9	42.2	40.2	42.9	42.3
8a	129.4	128.6	129.5	128.4	128.8
8b	120.6	120.0	120.7	120.0	119.7
1'	26.9	27.2	27.1	21.3	29.1
2'	129.1	125.9	129.0	8.5	25.2
3'	131.8	133.7	131.8	–	31.1
4'	30.7	30.9	30.7	–	33.1
5'	23.9	24.0	24.0	–	23.8
6'	14.3	14.3	14.3	–	14.5
1''	19.4	37.4	36.6	37.4	37.4
2''	–	21.6	21.8	21.4	21.4
3''	–	14.9	14.7	14.8	14.8

^{a)} Recorded at 125 MHz. ^{b)} Recorded at 150 MHz.

therefore different relative configurations. A NOESY experiment on **3** recorded in (D_7)DMF revealed that H–C(3a) ($\delta(\text{H})$ 4.23–4.26) and H–C(5a) (2.44–2.50) are located at the same side, whereas H–C(7) (1.83–1.88) and H–C(8) (2.38–2.41) appeared to be located at the opposite side of the molecule. The two side chains were therefore established to be *cis*, as in netamine O (**1**), but at the opposite side of the molecule compared to **1**. Additionally, as in netamines O (**1**) and P (**2**), the geometry of the C(2')=C(3') bond was assigned to be *cis* on the basis of the NOESY correlation between $\text{CH}_2(1')$ and $\text{CH}_2(4')$.

Netamine R (**4**) was isolated as pale yellow oil and showed the molecular composition of $\text{C}_{15}\text{H}_{26}\text{N}_3$, consistent with an ammonium salt with five C=C bond equivalents. Analysis of the 1D and 2D ^1H -, ^{13}C -, and ^{15}N -NMR data for **4** revealed resonances and correlations consistent with those of a tricyclic guanidine with a C(8a)=C(8b) bond. The HMB correlations for **4** clearly indicated that the heterocyclic ring system is substituted at C(7) ($\delta(\text{C})$ 37.7) and C(8) (42.9) by a Pr and an Et group, resp. The position of the Pr group at C(7) was confirmed by COSY correlations between $\text{CH}_2(1'')$ and H–C(7), and HMB correlations of Me(3'') with C(2'') and C(1''). Likewise, the location of the Et group at C(8) was confirmed by the COSY correlations Me(2'')/ $\text{CH}_2(1')$ and $\text{CH}_2(1')/\text{H–C}(8)$. The absence of NOE correlations prevented the determination of the configuration of **4**. The latter was deduced from empirical comparison of the chemical shifts of H–C(3a), H–C(5a), H–C(7), and H–C(8) belonging to **4** with those of **1**–**3** and **6**. Tables 2 and 3, set up for the purpose, showed that chemical shifts of the four CH H-atoms of netamine R were close to those of netamine P (**2**). The chemical shifts were also close to those of netamine E (**6**) [9][10].

Thus, it was finally assumed that, as for **2** and **6**, H–C(3a), H–C(5a), and H–C(7) are at the same side and H–C(8) is located at the opposite side of the molecule in **4**. The two side chains are therefore *trans*-oriented.

Netamine S (**5**) was isolated as brown oil. The molecular formula $C_{19}H_{34}N_3$ was deduced from the HR-ESI-MS and spectroscopic data. Netamine S (**5**), with two additional H-atoms compared to netamine P (**2**), was found to be the 2',3'-dihydro analog of **2**. The NOESY correlations H–C(3a) ($\delta(H)$ 4.25–4.30)/H–C(5a) (2.37–2.45) and H–C(5a)/H–C(7) (1.54–1.60) confirmed the location at the same side of the molecule for these three H-atoms. No correlation was observed between these three H-atoms and H–C(8) ($\delta(H)$ 1.92–1.97), excluding placement of H–C(8) at the same side of the molecule.

Biological Studies. The crude extract of the *B. laboutei* sponge showed potent cytotoxic activity against the KB tumor cell line (96.9% inhibition at 10 μM concentration). Netamines O–Q isolated in sufficient quantities were therefore evaluated for their cytotoxic activities against KB cells. Netamines O and Q were cytotoxic in the range of 10^{-5} M. Among the known guanidine compounds bearing a tricyclic 5,6,8b-triazaperhydroacenaphthylene skeleton, cytotoxic activities against cancer cell lines were also observed for ptilocaulin ($\Delta^{8,8a}$), isoptilocaulin ($\Delta^{6,7}$), mirabilin C acetate (pyrimidine), mirabilins F, G, and I ($\Delta^{8,8a}$), mirabilins H and K ($\Delta^{8a,8b}$), and netamines C and D (saturated) [1][4][8][9]. The cytotoxic activity of a synthetic racemic ptilocaulin was studied by *Rubent et al.* [12] in order to evaluate its *in vitro* activities against various cell lines. Moreover, the authors have shown that ptilocaulin was toxic at 50 and 25 mg kg⁻¹ in an *in vivo* L1210 tumor model and was ineffective at lower concentrations (T/C 100–112%).

The crude extract of the *B. laboutei* sponge also displayed promising *in vitro* antiparasmodial activity (IC_{50} 3.26 ± 0.25 μM). Thus, the *in vitro* activity of netamines O–Q was undertaken against *Plasmodium falciparum*. All other compounds were not isolated in sufficient quantities. Netamines O–Q exhibited antimalarial activities with IC_{50} values of 16.99 ± 4.12 , 32.62 ± 3.44 , and 8.37 ± 1.35 μM , respectively (Table 4). Four other tricyclic alkaloids have been reported as being active against the malaria parasite *P. falciparum*: a mixture of 1,8a-/8b,3a-dihydro-8 β -hydroxyptilocaulin and 1,8a-/8b,3a-dihydro-8 α -hydroxyptilocaulin (pyrimidine) with an IC_{50} value of 3.8 μg ml⁻¹ [3], netamine K, and mirabilin A with IC_{50} values of 2.4 and 20.7 μM , respectively.

Table 4. Antiplasmodial Activities of the Crude Extract of *B. laboutei* and of Netamines O–Q (**1–3**, resp.) against *P. falciparum*

Crude extract and pure compounds	IC_{50} [μM] ^{a)}
Crude extract	3.26 ± 0.25
1	16.99 ± 4.12
2	32.62 ± 3.44
3	8.37 ± 1.35
Artemisinin ^{b)}	0.014 ± 0.004
Chloroquine ^{b)}	0.050 ± 0.034

^{a)} The results were the average means of three replicate determinations \pm SD. ^{b)} Positive control.

Conclusions. – Five new tricyclic guanidine alkaloids, netamines O–S (**1–5**; resp.), together with the known compounds netamine E (**6**) and mirabilin J (**7**), were isolated from the sponge *B. laboutei*. Netamines O–Q, isolated in sufficient quantities, were evaluated for their cytotoxic and antiplasmodial activities. Netamines O and Q exhibited moderate cytotoxic activities. Netamines O–Q exhibited good antiplasmodial activities.

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Experimental Part

General. All solvents were anal. or HPLC grade and were used without further purification. Thin layer chromatography (TLC): precoated silica gel 60 F_{254} sheets (SiO_2); visualized by UV light at 254 nm and by spraying with vanillin/ H_2SO_4 followed by heating. MPLC: *Teledyne Isco CombiFlash® Companion®* with a *RediSep* preppacked normal-phase column (120 g) and *Büchi* system, including two C-605 pumps, a C-615 pump manager, a C-660 fraction collector, and a glass column (36 × 46 mm) packed with *Macherey–Nagel MN Kieselgel* (70–230 μm). Anal. HPLC: *Kinetex* (4.6 × 100 mm, 5 μm) or *Waters SunFire* (4.6 × 150 mm, 5 μm) column; *Waters 2695 Alliance* system equipped with a photodiode array detector (*Waters 996*), an evaporative light scattering detector (*Waters 2420*), and a mass spectrometer (*Waters Micromass ZQ 2000*). Prep. HPLC: *Waters SunFire Prep reversed-phase C₁₈* (RP-C₁₈) column (19 × 150 mm, 5 μm); *Waters 600* system controller equipped with a photodiode array detector (*Waters 2996*). Optical rotations: *MCP 300 Modular Circular Polarimeter Anton Paar* at 25°. UV Spectra: *Varian Cary* spectrometer; in MeOH; λ_{max} (log ϵ) in nm. IR Spectra: *PerkinElmer Spectrum 100* FT-IR spectrometer model instrument; dry film; $\tilde{\nu}$ in cm^{-1} . ^1H - and ^{13}C -NMR spectra: *Bruker UltraShield Avance-300*, –500, and *DRX-600* spectrometers; δ in ppm rel. to solvent signals ($\delta(\text{H})$ 3.31 and $\delta(\text{C})$ 49.15 for CD_3OD ; $\delta(\text{H})$ 8.03 and $\delta(\text{C})$ 163.00 for (D_7)DMF), J in Hz. The spectra were processed using 1D- and 2D-NMR notebook software. NOESY or ROESY spectra were recorded in (D_7)DMF or CD_3OD (see the Supporting Information¹⁾). CDCl_3 was used only for comparison with known compounds. HR-ESI-MS: *LCT Premier XE Micromass* spectrometer; in m/z .

Animal Material. The sponge *B. laboutei* HOOPER, 1996 (phylum, Porifera; class, Demospongiae; order, Poecilosclerida; family, Desmacellidae), identified by *J. V.*, was collected in October 2009 at four sampling stations in Salary Bay, Madagascar: station 1 (22°31'727" S, 43°13'597" E, at 18 m depth), station 2 (22°30'952" S, 43°12'558" E, at 25–30 m depth), station 3 (22°31'988" S, 43°13'036" E, at 30 m depth), station 4 (22°31'822" S, 43°12'939" E, at 25–27 m depth). Seven voucher specimens (# MHN.16242.1, MHN.16242.2, MHN.16242.3, MHN.16242.4, MHN.16242.5, MHN.16242.6, and MHN.16242.7) were deposited with the Museum d’Histoire Naturelle de Marseille, Palais Longchamp, 1 Bd Philippon, FR-13004 Marseille. Sponge samples were frozen immediately and kept at –20° until processing.

Extraction and Isolation. The $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 1:1 (3 × 3.5 l, each 24 h) extract (87 g) was obtained by maceration of the freeze-dried sponge (358 g) at r.t. The extract was then subjected to MPLC (SiO_2 ; isohexane/AcOEt/MeOH with increasing polarity) to give four fractions, *Fr. 1–4*. *Fr. 1* was eluted with isohexane/AcOEt 95:5, *Fr. 2* was eluted with isohexane/AcOEt 85:15, *Fr. 3* was eluted with AcOEt, and *Fr. 4* was eluted with AcOEt/MeOH 70:30.

¹⁾ Supporting material is available upon request from the authors.

Separation of *Fr. 4* (2.6 g) by MPLC (*RP-C₁₈*; H₂O/MeOH (+0.1% HCOOH)) gave twelve subfractions, *Fr. 4.1–4.12*. Anal. HPLC (*Waters SunFire C₁₈* (4.6 × 150 mm, 5 μm); 45% MeOH/H₂O (+0.1% HCOOH) to 70% MeOH/H₂O (+0.1% HCOOH), 1 ml min^{−1} gradient elution over 45 min; UV 254 nm, ELS) analyses showed that the isolated compounds are present in *Fr. 4.5* (108.8 mg) and *4.10* (150.0 mg). *Fr. 4.5* was subjected to semi-prep. HPLC (*Waters SunFire RP-C₁₈* (19 × 150 mm, 5 μm); 55% MeOH/H₂O (+0.1% HCOOH) to 68% MeOH/H₂O (+0.1% HCOOH), 4.8 ml min^{−1} gradient elution over 20 min; UV 254 nm) to furnish four subfractions, *Fr. 4.5.1–4.5.4*, containing pure compound **7** (mirabiline J; 5.5 mg). *Fr. 4.10* was subjected to subsequent semi-prep. HPLC (*Waters SunFire RP-C₁₈*; 75% MeOH/H₂O (+0.1% HCOOH), 5 ml min^{−1} isocratic elution over 15 min; UV 254 nm) to give five subfractions, *Fr. 4.10.1–4.10.5*. Two of them, *Fr. 4.10.2* and *4.10.3*, afforded pure compounds **6** (netamine E; 4.0 mg) and **3** (netamine Q; 8.9 mg).

Fr. 4 (360 mg) was also subjected to prep. HPLC (*Waters SunFire Prep RP-C₁₈* (19 × 150 mm, 5 μm); 45% MeOH/H₂O (+0.1% HCOOH) to 70% MeOH/H₂O (+0.1% HCOOH), 17 ml min^{−1} gradient elution over 45 min; UV 254 nm) to give 13 subfractions, *Fr. 4.1'–4.13'*. One of them afforded **1** (netamine O; 8.4 mg). Three other subfractions were subjected to subsequent prep. HPLC (*Waters SunFire Prep RP-C₁₈*; 55% MeOH/H₂O (+0.1% HCOOH) to 70% MeOH/H₂O (+0.1% HCOOH), 17 ml min^{−1} gradient elution over 26 min; UV 254 nm) to give pure compounds **2** (netamine P; 3.9 mg), **4** (netamine R; 1.9 mg), and **5** (netamine S; 1.9 mg).

Netamine O (= (3*aS**,5*aS**,7*R**,8*R**)-8-[(2*Z*)-Hex-2-en-1-yl]-3,3*a*,4,5,5*a*,6,7,8-octahydro-7-methylcyclopenta[de]quinazolin-2-amine; **1**). Yellow oil. [α]_D²⁵ = +61.6 (*c* = 1.33, MeOH). UV: 234 (0.95), 302 (0.28). IR: 2990, 1599. ¹H- and ¹³C-NMR: see Table 1. HR-ESI-MS: 274.2271 (*M*⁺, C₁₇H₂₈N₃⁺; calc. 274.2278).

Netamine P (= (3*aS**,5*aS**,7*R**,8*S**)-8-[(2*Z*)-Hex-2-en-1-yl]-3,3*a*,4,5,5*a*,6,7,8-octahydro-7-propylcyclopenta[de]quinazolin-2-amine; **2**). White solid. [α]_D²⁵ = +219.0 (*c* = 1.46, MeOH). UV: 235 (0.56). IR: 3290, 1646. ¹H- and ¹³C-NMR: see Tables 2 and 3. HR-ESI-MS: 302.2600 (*M*⁺, C₁₉H₃₂N₃⁺; calc. 302.2591).

Netamine Q (= (3*aS**,5*aS**,7*S**,8*S**)-8-[(2*Z*)-Hex-2-en-1-yl]-3,3*a*,4,5,5*a*,6,7,8-octahydro-7-propylcyclopenta[de]quinazolin-2-amine; **3**). Yellow oil. [α]_D²⁵ = +18.3 (*c* = 0.30, MeOH). UV: 233 (0.94), 305 (0.17). IR: 3300, 1648. ¹H- and ¹³C-NMR: see Tables 2 and 3. HR-ESI-MS: 302.2578 (*M*⁺, C₁₉H₃₂N₃⁺; calc. 302.2591).

Netamine R (= (3*aS**,5*aS**,7*R**,8*S**)-8-Ethyl-3,3*a*,4,5,5*a*,6,7,8-octahydro-7-propylcyclopenta[de]quinazolin-2-amine; **4**). Pale yellow oil. [α]_D²⁵ = +18.0 (*c* = 0.10, MeOH). UV: 238 (0.63). IR: 3300, 1650. ¹H- and ¹³C-NMR: see Tables 2 and 3. HR-ESI-MS: 248.2146 (*M*⁺, C₁₅H₂₆N₃⁺; calc. 248.2121).

Netamine S (= (3*aS**,5*aS**,7*R**,8*S**)-8-Hexyl-3,3*a*,4,5,5*a*,6,7,8-octahydro-7-propylcyclopenta[de]quinazolin-2-amine; **5**). Pale brown oil. [α]_D²⁵ = +20.0 (*c* = 0.10, MeOH). UV: 248 (0.30). IR: 3300, 1650. ¹H- and ¹³C-NMR: see Tables 2 and 3. HR-ESI-MS: 304.2748 (*M*⁺, C₁₉H₃₄N₃⁺; calc. 304.2747).

Netamine E (= (3*aS*,5*aS*,7*R*,8*S*)-8-Butyl-3,3*a*,4,5,5*a*,6,7,8-octahydro-7-propylcyclopenta[de]quinazolin-2-amine; **6**). Yellow oil. [α]_D²⁵ = +37.0 (*c* = 0.10, CH₂Cl₂). UV: 238 (0.63). HR-ESI-MS: 276.2441 (*M*⁺, C₁₇H₃₀N₃⁺; calc. 276.2434).

Mirabilin J (= (3*aS*,5*aS*,7*R*,8*S*)-2-Amino-8-[(2*Z*)-hex-2-en-1-yl]-3,3*a*,4,5,5*a*,6,7,8-octahydro-7-methylcyclopenta[de]quinazolin-8-ol; **7**). Brown oil. [α]_D²⁵ = +78.0 (*c* = 0.18, CH₂Cl₂). HR-ESI-MS: 290.2238 (*M*⁺, C₁₇H₂₈N₃O⁺; calc. 290.2227).

In vitro Cytotoxicity Assay Against KB Cell Line. Cell proliferation was measured with Celltiter 96 Aqueous One soln. reagent (Promega) and results are expressed as the percentage of inhibition of cellular proliferation of KB cells treated for 72 h with **1–3** compared to cells treated with DMSO only (mean ± SE of triplicate). The IC₅₀ determinations were performed in duplicate experiments and are expressed as individual values.

In vitro Antiplasmodial Assays. *P. falciparum* strains were utilized and details of the assay protocols have been previously reported [13]. Artemisinin (98%; Sigma–Aldrich) and chloroquine (>98%; Sigma–Aldrich) were used as positive controls.

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